

Occurrence and Intra-specific Variation of Sweetpotato Weevil (Brentidae: Coleoptera) in Relation to Its Potential Spread in Southern United States of America and the Caribbean

Louis EN Jackai¹, Bryon Sosinski², D. Michael Jackson³, Kenneth A. Sorensen², Conrad K. Bonsi¹, Abraham Addo-Bediako¹, Regina Ali², B. Tameru¹, Franklin Quarcoo¹ and Marikis N. Alvarez¹

¹Tuskegee University, Tuskegee, Alabama, USA

²North Carolina State University, Raleigh, North Carolina, USA

³USDA-ARS, USA

Keywords: DNA fingerprinting, pest profiles, risk assessment, environmental effects, GIS

Abstract

The sweetpotato weevil, *Cylas formicarius*, causes severe damage to roots and other parts of the sweetpotato crop in the field as well as in storage, resulting in losses worth millions of dollars in revenue and food calories each year around the world. In the U.S.A., quarantine measures are in force in most southern States where the weevil is a problem. However, this has not stopped the spread of the weevil, nor the crop devastation it causes. In a USDA-funded project we have begun to study the factors that lead to weevil spread to non-infested areas and the genetic variation within the *Cylas formicarius* populations in the U.S. and the Caribbean. Preliminary results show that sufficient polymorphism exists among weevil populations to suggest important differences in these populations. Risk analysis studies also show the probability of weevil introduction into a new area without any mitigation measure to be 0.2667; that of weevil introduction following a pre-harvest mitigation effort was determined as 0.1175, and that of introduction following post-harvest mitigation 0.000517. The calculated overall risk of weevil introduction was 0.0000162, a low but nonetheless important risk level in a country where a single weevil could lead to the rejection of an entire crop and the imposition of strict quarantine measures with severe economic impact to growers. There are locations where no weevil infestations have ever been reported; GIS-aided studies will determine the environmental conditions that foster weevil establishment and thus provide a means of identifying "high risk" areas for weevil spread. We propose the formation of a network to conduct similar studies in other parts of the world.

INTRODUCTION

Sweetpotato production in the United States of America (U.S.) ranks tenth worldwide, but that is nowhere near where it could be. In 2004, 720,900 tonnes were produced (cf. 106,197,100 tonnes in China, the leading producer) (FAOSTATS, 2005: <http://faostat.fao.org/faostat/notes/citation.htm>). Among the reasons for the relatively low production of sweetpotato in the U.S. are several insect pests, among which the sweetpotato weevil (SPW), *Cylas formicarius* Fab., is the most devastating. This is an important pest of sweetpotato worldwide (Wolfe, 1991). In the U.S., damage by *Cylas formicarius elegantulus* (Summers) costs the sweetpotato industry several million dollars annually. Many growers in highly infested areas routinely use various field insecticides, and bromide to fumigate their sweetpotato, especially in storage prior to marketing; others have simply abandoned growing sweetpotato. Although the U.S. remains a net exporter of sweetpotato (\$14 million in exports in 2001), sweetpotato worth \$5.1 million were imported in 1999/2000 mostly from the Dominican Republic directly into Puerto Rico (Lucier et al., 2002; USDA-FAS: <http://www.census.gov/foreign-trade/>). There are a number of important questions that need to be answered in order to improve management of the SPW. Among these are: "Why does this pest continue to spread so readily (new

infestations are identified every year), and what may be the risk posed by this trend? Are there ecotypes present in the U.S. and Caribbean weevil populations? What environmental conditions foster weevil survival?" Our project seeks to find answers to these questions in order to increase our understanding of the environmental relationships between the insect, the crop and human activity and movement, genetics and management of this weevil. The project focuses on the modalities through which the SPW spreads from infested to non-infested areas, and also investigates the likely existence of intra-specific variation among SPW from different parts of the region, especially given that this species has a circumglobal distribution (Wolfe, 1991; CAB International, 2002). In this paper, we outline the progress made so far towards this goal in a USDA-funded project, and we suggest the steps that may be needed to combat this pest on a global basis.

RESEARCH APPROACH

A multidisciplinary team comprising university and government scientists from Tuskegee University (TU) (the lead institution), North Carolina State University (NCSU), USDA-ARS in Charleston, South Carolina, and a number of other individuals in academia, research and State Departments of Agriculture collaborated in this project. The weevil DNA work was conducted in NCSU, while pest profile studies, pest risk analysis (PRA), GIS and the economic analysis work was at TU. The group met once a year to review progress, but there was constant electronic communication among members of the group. The project is in its final phase; therefore, only preliminary findings will be reported here.

MATERIALS AND METHODS

Development of Protocols

Two different protocols were developed, one for the collection of insects and the other for DNA analysis. The weevil collection protocol ensured that the insects were collected alive using Universal[®] Traps (supplier - Great Lakes IPM) and lure for the SPW that were provided by the project. Each trap had a small sweetpotato in place of the usual killing strip. Twenty to 50 weevils collected from up to 50 different sites in southern USA and the Caribbean that were at least 50 miles apart were shipped in 70% ethanol to NCSU following quarantine clearance obtained from the North Carolina Department of Agriculture. Each collection site was identified by GIS coordinates obtained using GPS hardware provided by the project.

The protocol for DNA extraction focused on the 18SrRNA gene which has been sequenced in *C. formicarius* and other weevil species, and documented in the National Center for Biotechnology Information (NCBI) database. Multiple alignments of the published sequences were performed to identify regions of the gene that were likely to yield usable polymorphisms both between and within *Cylas*. Two sets of primers, each flanking a separate region of high variability between species, were chosen for PCR and subsequent DNA sequencing. All four primers were further evaluated for consistency of amplification, and for ease of sequencing, and a single set of primers was selected for further studies. A number of different DNA extraction protocols were evaluated (Votkin, Gentra, Qiagen, and cetyltrimethylammonium bromide or CTAB) and their variations noted. Initial DNA yields were inconsistent with the majority of the protocols, often with signs of degradation, but the QIAGEN protocol (DNeasy kit, animal protocol) was found to be the most consistent and is now routinely employed. PCR amplifications were performed as reported by Lewis et al. (2002). Weevils were analyzed for sequence polymorphism by multiple alignment using Vector NTI (Invitrogen).

Pest Census on Sweetpotato Cultivars

Two locations in Alabama, one infested with the SPW (Fairhope, Baldwin County), and the other weevil-free (Tuskegee, Macon County), were used for field studies in which four sweetpotato cultivars ('Beauregard', 'Hernandez', 'W-308' and '606258')

were grown and monitored for pest incidence using sticky cards and Universal Traps equipped with SPW pheromone. Data were collected on the insect pest profiles (SPW and others) and root yield. These data along with the GIS information will be used to determine the role of the environment in the incidence and distribution of the weevil, and possibly to provide a basis for predicting its spread. GIS is an ideal tool for managing data about the nature, location and spread of pests (Williams, 2004).

Pest Risk Analysis

A risk analysis of the weevil in southern USA and the Caribbean as conducted following traditional pathway analysis (Palisade Corporation, 2003) to estimate the probability that the SWP from an infested area can be introduced to non-infested areas within the southern United States. Pathway analysis relies on an accurate definition of events (steps) on the pest movement from source to destination. Each event involves a critical change in the presence of the pest, typically a reduction in the probability of its continued transfer along the pathway conditioned by either its biology or a management intervention (Auclair et al., 2005). Using published and unpublished but accessible information as well as personal communication with researchers, farmers and others, the risk analysis study was initiated following established qualitative and quantitative methods and tools. The impact of pre- (with chlorpyrifos or endosulfan applied in a two-week cycle during crop growth) and post- mitigation (with imidan 5% dust applied before storage, and legislation controlling movement of roots and cuttings) treatments on weevil movement was assessed. Upon the completion of the pest risk assessment, an economic analysis will determine the cost of the various mitigation options as well as the impact of the weevil in southern USA.

PRELIMINARY FINDINGS

Protocol Development

Although the protocol for weevil collection seemed straightforward, some modifications became necessary after the first year of specimen collection when we discovered that some of the weevils sent for DNA analysis had been collected already dead and were thus unsuitable for DNA extraction.

The modified protocol provided better specimens for subsequent DNA extractions. It was also determined that the extractions could only effectively be performed using the excised head of the insect. Following sequencing optimization, each sample was tested for different experimental conditions including amplification settings, primer and DNA template concentrations, and others. Results indicate that all primer settings worked reasonably well for sequencing. Minor variations were observed in the quality of the sequence. For example, an annealing temperature of 56° or 57°C gave the cleanest, most specific band of the product when compared with other temperatures. The Qiagen PCR purification methods gave better DNA yield and quality than ethanol precipitation. The primer and DNA concentrations seemed to have less effect than the annealing temperature and clean-up at the ranges tested. As a result of initial unsatisfactory AFLP results, the focus was turned to ribosomal genes using published weevil and/or coleopteran sequences. Primers were designed in conserved regions with very encouraging results (Fig. 1). Although still preliminary, these results are indicative of the presence of sufficient polymorphism to suggest differences in the weevil populations. Further details will be reported in subsequent papers. Figure 2 illustrates the high level of relatedness of the weevils studied thus far. It is interesting to note that the sequences analyzed place the published sequence as an outlier as compared to the study samples. Samples collected from Charleston SC (#s 5-9), Florida (#s 12-26) and Lee County Alabama (#11) all show high similarity to samples collected from the Caribbean, while the weevils from an area adjacent to Tuskegee appear to be sequence diverged. Additional studies are underway to confirm these preliminary results.

Pest Census on Sweetpotato Cultivars

No weevils were trapped at Tuskegee, but some were found in Fairhope. The index of pest diversity was more than twice higher in Fairhope than at Tuskegee, suggesting many more insect species at the former location which is a short drive from Alabama's Gulf Coast. In addition, certain pest species encountered in fairly high numbers in Fairhope were either not observed, or were present in very low numbers in Tuskegee, among them are the banded cucumber beetle (*Diabrotica balteata* Le Conte), and the sugarcane beetle (*Euethola humilis rugicipeps* LeConte). Overall pest census on all the four test cultivars was similar but only W-308 which was bred for resistance to the weevil showed little or no damage; others had significant root damage (Table 1).

Pest Risk Analysis

Pest Risk Analysis studies have identified, inter alia, the established and pre-defined pathways for weevil introduction (Table 2), and produced a compilation of the most recent reports of weevil infestation in some states in southern U.S. (Table 3). The probability that a weevil will be introduced into a new area without any mitigation measure was shown to be 0.2667; the probability of weevil introduction following pre-harvest mitigation which was determined as 0.1175; while that of introduction following post-harvest mitigation was judged to be 0.000517 (or $\ll 0.1\%$). With a calculated overall risk of weevil introduction using @RISK Software (Palisade Corp., 2003) was 0.0000162 (around 0.002%) one may be tempted to underestimate the potential for spread and the part this plays in the overall SPW problem. The final outcome as to how successfully the weevil gets established is a function of the survival of introduced weevils and the likelihood of interception at the points of entry (Table 4) into a non-infested area. Much work remains to be done in this area, but it is important to realize that it takes only one pair of weevils, or a single gravid female, to start an outbreak, and the economic implications for the U.S. grower can be overwhelming.

CONCLUSION AND FUTURE DIRECTIONS

The findings from this project will hopefully lead to the further elucidation of the pathways (routes) used in the spread of the SPW, and the determination of the existence of intra-specific variation in the SPW population in the USA and Caribbean. These outcomes will lead to more effective and targeted management of the SPW, taking into consideration those pathways and interactions that were either unknown, or under-emphasized by state quarantine regulations. This would enhance quarantine enforcement and reduce the risk of weevil spread.

Given the global importance of SPW and *C. formicarius* in particular, it is necessary to extend this work to other regions of the world, in particular Africa and Asia, both of which have more than one weevil species. This might be achieved through the creation of a research network on the SPW with a focus on PRA and genetic variation and origins. The information generated will be useful in the development of location/region-specific management strategies (including resistant cultivars, biological and others). Management of the SPW not only requires a multi-mechanistic approach but also a new paradigm that utilizes knowledge of pest risk analysis, GIS and molecular taxonomy. In the long run, we hope this project could serve as a model for the control of other highly invasive pests that could be managed globally after the requisite knowledge has been accumulated.

ACKNOWLEDGEMENTS

This study was funded by a Capacity Building Grant from USDA-CSREES.

Literature Cited

- Auclair, A.N.D., Fowler, G., Hennessey, M.K., Hogue, A.T., Keena, M., Lance, D.R., McDowell, R.M., Oryang, D.O. and Sawyer, A.J. 2005. Assessment of the risk of introduction of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in municipal

- solid waste from the quarantine area of New York City to landfills outside of the quarantine area: A pathway analysis of the risk of spread and establishment. *J. Econ. Entomol.* 98:47-60.
- CAB International, 2002. *Crop Protection Compendium, Global Module*, 4th ed. Wallingford, UK: CAB International.
- FAOSTATS. 2005. <http://faostat.fao.org/faostat/notes/citation.htm> (accessed 8 June 2005).
- Lewis K.G., Liewlaksaneeyanawin, C., Alfaro, R.I., Ritland, C., Ritland, K. and El-Kassaby, Y.A. 2002. Sexual reproduction in the white pine weevil (*Pissodes strobi* [Peck] [Coleoptera: Curculionidae]): Implications for population genetic diversity. *The J. Heredity* 93(3):165-169.
- Lucier, G, Allshouse, J. and Lin, B.H. 2002. Sweet potatoes: Getting to the root of demand. *Agri. Outlook*, Nov. 2002, ERS-USDA:3-17.
- NAPIS. 2004. <http://www.ceris.purdue.edu/napis/states> (accessed 30 July 2004). Palisade Corporation. 2003. *@Risk 4.5*, Palisade Corporation, Newfield, NY.
- USDA-FAS. <http://www.census.gov/foreign-trade/> (accessed 31 January 2005)
- Williams, J. 2004. Innovative Uses of GIS with GPS in Pest Control. [http://gis.esri.com/library/userconf/proc0/professional/pap375.ht](http://gis.esri.com/library/userconf/proc0/professional/pa....p375.ht). (accessed 22 January 2004).
- Wolfe, G.M. 1991. The origin and dispersal of the pest species of *Cylas* with a key to the pest species groups of the world. p. 13-43, In: R.K. Jansson and K.V. Raman (eds.), *Sweet Potato Pest Management: A global perspective*. Westview Press. Boulder, Colorado. 458 p.

Tables

Table 1. Percentage of roots damaged¹ by the sweetpotato weevil in an experimental plot in Fairhope, Baldwin County, Alabama, USA.

Cultivar	Percentage roots with exit holes	Percentage roots with internal damage
W-308	18.3	0.0 ²
606258	64.6	10.0
Beauregard	56.9	60.0
Hernandez	66.2	20.0

¹Based on a sample of ten roots per replication (n=40)

²Only superficial damage observed

Table 2. Pathways of movement of the sweetpotato weevil in southern United States.

Established pathways	Re-defined pathways
1. Permitted cargo from infested to non-infested areas	1. Innocent transport by non-suspecting relations, friends or new garden farmers
2. Cargo ships (e.g. from the Caribbean)	2. Movement of ornamentals/alternative hosts
3. Vendors	3. Wind-aided dispersal (e.g. during hurricanes)
4. Natural spread	4. Research activities
5. Air passengers and baggage from foreign areas	5. Movement as hitchhikers
6. Possible variations from quarantine seed certificates	

Table 3. New reports of the sweetpotato weevil in southern USA¹. (Source; Addo-Bediako et al., Tuskegee Univ., 2005. unpublished data)

State	Number of new occurrences	Reason for establishment failure	Source of information
Alabama	2	Agriculture practice	T. Taylor, Alabama Dept. of Agric., pers. comm.
South Carolina	2	Quarantine	National Agricultural Pest Information System [NAPIS] (2004)
North Carolina	2	Quarantine	NAPIS (2004)
Louisiana	0	Agriculture practice	A. Hammond, LSU, pers. comm.
Mississippi	4	Quarantine	B. Graves, Mississippi Dept. of Agric., pers. comm.
Georgia	1	No record	NAPIS (2004)
Texas	1	Eradiation	S. Nilakhe, Texas Dept. of Agric., pers. comm.

¹New occurrences reported for the past five years; for Mississippi, for the past 10 years.

Table 4. Possible pathways for entry of the sweetpotato weevil used in the analysis.

Possible pathway	Rank
1. Permitted cargo moves from infested to non-infested areas, including hitchhikers	High
2. Vendors selling commodities, including ornamentals, from infested areas	High
3. Natural spread (e.g. wind-borne, hitchhikers, etc.)	Low
4. Planting materials from infested to non-infested areas	High

Figures

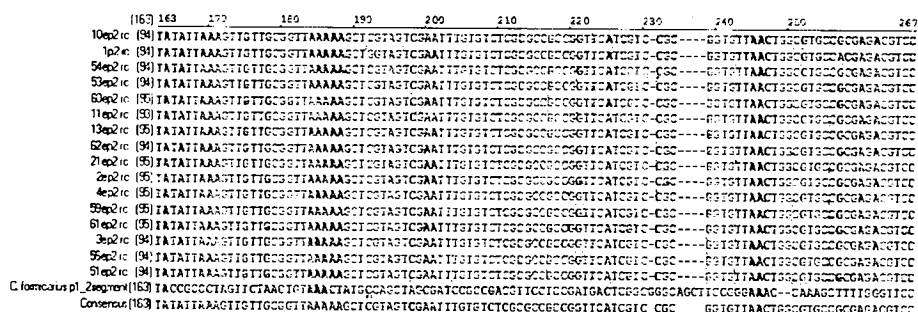


Fig. 1. Multiple alignment of 18S weevil sequences illustrating polymorphisms between the published sequence and between collection sites. Blue shading indicates sequence differences observed between the published weevil sequence and the test samples, while yellow indicates sequence homology.

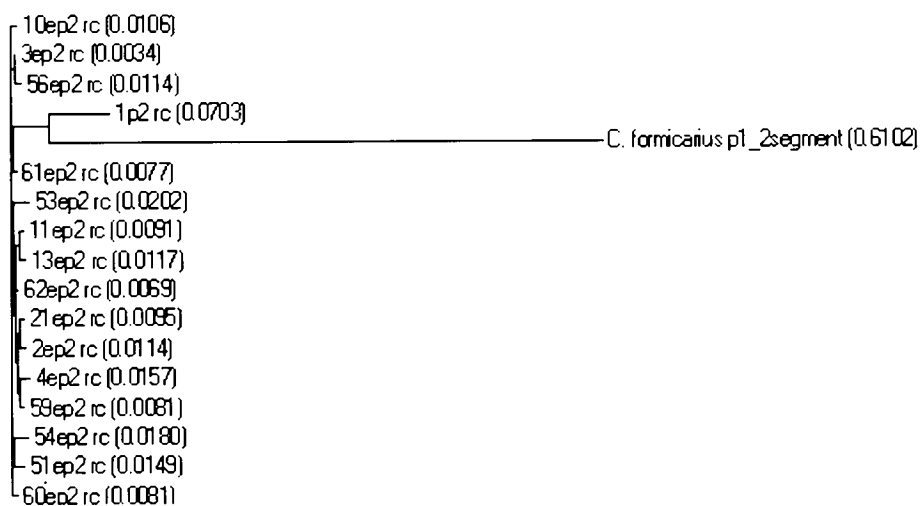


Fig. 2. Dendrogram illustrating overall relatedness of weevils from collection sites.